date of death, liver transplantation (LT), last contact, death from non-liver associated diseases, or the end of the follow-up period, whichever came first.

The demographic features of the 334 PBC patients at the time of entry are shown in Table 1. Among the 334 patients, 99 patients (29.6%) had the following concomitant autoimmune diseases: Sjogren's syndrome ($n = 43$), autoimmune hepatitis ($n = 25$), Hashimoto's thyroiditis ($n = 13$), rheumatoid arthritis ($n = 10$), Raynaud's disease ($n = 8$), systemic sclerosis ($n = 7$), CREST syndrome ($n = 4$), autoimmune thrombocytopenic purpura ($n = 3$), interstitial pneumonia ($n = 2$), hypothyroidism ($n = 2$), polymyositis ($n = 1$), systemic lupus erythematosus ($n = 1$), mixed connective tissue disease ($n = 1$), eosinophilic fasciitis ($n = 1$), uveitis ($n = 1$), and sarcoidosis ($n = 1$). Among these concomitant autoimmune diseases, 7 patients with AIH, 2 patients with Sjogren's syndrome, 2 patients with interstitial pneumonia, one patient with polymyositis, one patient with eosinophilic fasciitis were diagnosed during the course of observation.

Patients were treated for PBC during the observation periods with the following: ursodeoxycholic acid (300–900 mg/day) alone ($n = 213$), bezafibrate (200–400 mg/day) alone ($n = 4$), prednisolone (>20 mg/day) ($n = 19$), ursodeoxycholic acid + bezafibrate ($n = 70$), ursodeoxycholic acid and/or bezafibrate + maintenance prednisolone (<5 mg/day) ($n = 25$), or no medication ($n = 13$).

Enzyme-linked immunosorbent assay (ELISA)

Serum samples were serially taken, stored at −20°C at each institution, and then tested for autoantibodies by ELISA. At least two serum samples obtained at different times (2–25 samples, median 5, mean ± SD 6.2 ± 4.7) were used to measure autoantibodies over the observation period. Serum antibody titers to the gp210 C-terminal peptide a.a.1863–1887 (SPNALPPARKA SPPSGLWSP AYASH) were measured as described previously.[9] The antibody titers were calculated with reference to a standard serum, and titers more than 6 units/mL (mean value + 5 SD of the titers of 30 healthy controls) were arbitrary defined as antibody-positive. Antibody titers to mitochondrial antigens MIT3 (recombinant proteins containing PDC-E2, BCOADC-E2, OGDC-E2) and SP100 (synthetic peptides) were determined using ELISA kits (INOVA Diagnostics, San Diego CA), and antibody titers greater than 25 units/mL were considered positive according to the manufacturer's protocol.[11] Antibody titers to centromere B (recombinant centromere B proteins) were determined using the Mesacup-2 CENP-B ELISA kit (MBL, Nagoya) according to the manufacturer's instructions, and titers more than 25 units/mL were considered positive.[11]

DNA extraction and HLA genotyping

Genomic DNA was purified from the peripheral whole blood of PBC patients using a DNA isolation kit following the manufacturer's instructions (Macherey-Nagel, Düren). *HLA-DRB1* genotyping was performed as described previously.[32] Briefly, the *HLA-DRB1* genotype was determined by sequence-based typing (SBT) of group-specific PCR products. The nucleotide sequence of the *HLA-DRB1* locus was analyzed using Assign 400ATF software (Conexio Genomics, Applecross).

Ethics board

The present study was approved by the ethics board of each medical center affiliated with the NHOSLJ. All subjects gave informed consent to use their serum and DNA samples to advance medical knowledge on the causes of PBC.

Statistical analysis

The frequency of the HLA–DRB1 alleles in PBC patients was compared to the general Japanese population,[33] and the strength of the association was expressed as an odds ratio (OR). The statistical significance of this association was examined by Fisher's exact test included in the R statistical package (available online at http://www.r-project.org/). Fisher's exact test was also used to compare the frequency of cases between different *HLA-DRB1* alleles. An unconditional step-wise logistic regression analysis was used to determine the independent significance of the *HLA-DRB1* alleles, ANAs, sex, and age at the end of the observation period for the two different types of PBC progression (nonjaundice-type and jaundice-type progression). These statistical analyses were conducted using SAS, version 9.3 (SAS Institute, Cary NC). All statistical tests were based on two-sided probability, and *P*-values less than 0.05 were considered statistically significant.

RESULTS

Demographic features and clinical course of PBC patients during the observation period

UPON ENTERING THE study, 283, 49 and two PBC patients were in clinical stage I (early stage), clinical stage II (late stage without jaundice), and clinical stage III (late stage with jaundice), respectively

(Table 1). During the observation period (1–452 months, median 57, mean ± SD 69.6 ± 63.1), 36 and nine of the 283 patients initially in clinical stage I had progressed to clinical stage II and III, respectively. Seven out of the 49 patients initially in clinical stage II progressed to clinical stage III during the observation period. At the end of the observation period, 238, 78, and 18 patients were in clinical stage I, II, and III, respectively. Among the 18 clinical stage III patients, eight patients received a liver transplant, six patients died of end-stage hepatic failure without receiving a liver transplant, and four patients who did not receive a liver transplant were alive.

To determine the time course of autoantibody production, serum anti-gp210, anti-centromere, anti-sp100 antibodies and AMAs were serially measured at least twice (2–25 times, median 5, mean ± SD 6.2 ± 4.7) for each patient over the observation period. Nine out of 103 initially anti-gp210-positive patients became persistently anti-gp210-negative, whereas 13 out of 230 initially anti-gp210-negative patients became persistently anti-gp210-positive. The anti-centromere antibody status did not change from positive to negative or vice versa in all of the patients, but two of 40 initially anti-sp100-positive and 3 of 299 initially AMA-positive patients became anti-sp100- and AMA-negative, respectively, over the observation period. Thus, anti-gp210, anti-centromere, anti-sp100 antibodies and AMAs were positive in 107 (32.0%), 82 (24.6%), 38 (11.4%) and 296 (88.6%) patients, respectively, at the end of the observation period.

Frequency of *HLA-DRB1* alleles in PBC patients

As shown in Table 2, the frequency of the *HLA-DRB1*0803* and *-DRB1*0405* alleles was significantly increased in PBC patients compared to the general Japanese population (13.3% vs. 6.4%, OR, 2.24, 95% CI,

Table 2 Frequency of *HLA-DRB1* alleles in primary biliary cirrhosis patients

Allele	PBC ($n = 668$)	Control ($n = 516$)	*Odds ratio (95% confidence interval)	*P-value
*0101	32 (4.8%)	20 (3.9%)	1.24 (0.70, 2.20)	
*0301	1 (0.1%)	0 (0%)	2.32 (0.09, 57.1)	
*0401	6 (0.9%)	7 (1.4%)	0.66 (0.22, 1.97)	
*0403	17 (2.5%)	20 (3.9%)	0.65 (0.33, 1.24)	
*0404/23	1 (0.1%)	0 (0%)	2.32 (0.09, 57.1)	
*0405	126 (18.9%)	68 (13.2%)	1.53 (1.11, 2.11)	0.005
*0406	11 (1.6%)	18 (3.5%)	0.46 (0.21, 0.99)	
*0407	2 (0.3%)	2 (0.4%)	0.77 (0.11, 5.49)	
*0409	1 (0.1%)	0 (0%)	2.32 (0.09, 57.1)	
*0410	12 (1.8%)	11 (2.1%)	0.84 (0.36, 2.04)	
*0701	4 (0.6%)	4 (0.8%)	0.77 (0.19, 3.09)	
*0801	1 (0.1%)	0 (0%)	2.32 (0.09, 57.1)	
*0802	37 (5.5%)	18 (3.5%)	1.62 (0.91, 2.88)	
*0803	89 (13.3%)	33 (6.4%)	2.24 (1.48, 3.41)	0.0001
*0901	89 (13.3%)	71 (13.8%)	0.96 (0.69, 1.34)	
*1001	7 (1.0%)	3 (0.6%)	1.81 (0.46, 7.03)	
*1101	7 (1.0%)	19 (3.7%)	0.28 (0.11, 0.66)	0.002
*1201/06/10	14 (2.1%)	13 (2.5%)	0.83 (0.38, 1.77)	
*1202	6 (0.9%)	11 (2.1%)	0.41 (0.15, 1.13)	
*1301	2 (0.3%)	2 (0.4%)	0.77 (0.10, 5.49)	
*1302	15 (2.2%)	29 (5.6%)	0.38 (0.20, 0.72)	0.003
*1401/54	23 (3.4%)	22 (4.3%)	0.80 (0.44, 1.45)	
*1405	24 (3.6%)	16 (3.1%)	1.16 (0.61, 2.21)	
*1406	5 (0.7%)	11 (2.1%)	0.34 (0.12, 1.00)	
*1501	46 (6.9%)	60 (11.6%)	0.56 (0.37, 0.84)	0.005
*1502	81 (12.1%)	46 (8.9%)	1.41 (0.96, 2.06)	
*1602	9 (1.3%)	4 (0.8%)	1.74 (0.53, 5.70)	

*Fisher's exact test.

Table 3 Autoantibodies as a risk factor for disease progression in primary biliary cirrhosis

Variables	*Odds ratio (95% CI) for progression to Clinical stage II	Clinical stage III
Sex, male		
Age (one year – 1)	1.05 (1.02, 1.08)	0.94 (0.90, 0.99)
Anti-gp210-positive	3.69 (2.05, 6.64)	46.56 (9.20, 850.15)
Anti-centromere-positive	2.36 (1.28, 4.35)	
Anti-sp100-positive		

*Odds ratio was calculated using an unconditional step – wise logistic regression analysis.

1.48–3.41, p = 0.0001 and 18.9% vs. 13.2%, OR, 1.53, 95% CI, 1.11–2.11, $P = 0.005$, respectively), whereas the frequency of HLA-DRB1*1101, -DRB1*1302, and -DRB1*1501 was significantly decreased in PBC patients compared to the general Japanese population (1.0% vs. 3.7%, OR, 0.28, 95% CI, 0.11–0.66, $P = 0.002$; 2.2% vs. 5.6%, OR, 0.38, 95% CI, 0.20–0.72, $P = 0.003$ and 6.9% vs. 11.6%, OR, 0.56, 95% CI, 0.37–0.84, $P = 0.005$, respectively).[33]

All of these *P*-values remained significant even after these values were corrected based on the Bonferroni inequality method; a correction factor of 10, which represents the total number of detected alleles that constitute more than 3.7% of the total *HLA-DRB1* alleles in the control Japanese population, was applied. There was no significant difference in demographic features (i.e. sex, age, clinical stage, positivity of ANAs and AMAs) between the PBC-susceptible alleles (i.e. *HLA-DRB1*0405* and **0803*) and PBC-resistant alleles (i.e. *HLA-DRB1 *1101, *1302, *1501*; data not shown).

ANAs as a significant risk factor for the disease progression in PBC

Age and the ANA status at the end of observation were used to analyze the risk factors associated with PBC disease progression. The presence of anti-gp210 antibodies was a strong risk factor for jaundice-type progression (OR, 46.56, 95% CI, 9.20–850.15), while anti-centromere antibodies was a significant risk factor for nonjaundice-type progression (OR, 2.36, 95% CI, 1.28–4.35), which confirmed our previous observations (Table 3).[11] In addition, anti-gp210 antibody seropositivity was also a significant risk factor for nonjaundice-type progression (OR, 3.69, 95% CI, 2.05–6.64), whereas anti-sp100 antibody seropositivity was not a significant risk factor for any type of PBC disease progression (Table 3). Age was also a positive and negative risk factor for nonjaundice-type (OR, 1.05, 95% CI, 1.02–1.08) and jaundice-type (OR, 0.94, 95% CI, 0.90–0.99) progression, respectively (Table 3).

The association of *HLA-DRB1* alleles with ANA production and disease progression

The association of *HLA-DRB1* polymorphisms with ANA production and disease progression was analyzed using data on the *HLA-DRB1* alleles, and those with an allelic frequency greater than 5% in PBC patients were

Table 4 The association of *HLA-DRB1* alleles with autoantibody production and disease progression in primary biliary cirrhosis

Variables	*Odds ratio (95% confidence interval)			
	Antibodies-positive for: Anti-gp210	Anti-centromere	Progression to Clinical stage II	Clinical stage III
Sex, male				
Age (one year-1)		1.05 (1.03, 1.07)	1.06 (1.04, 1.08)	0.95 (0.93, 0.98)
*HLA-DRB1*0405*	1.61 (1.08, 2.39)			
*HLA-DRB1*0803*		2.30 (1.41, 3.73)		
*HLA-DRB1*0901*			1.78 (1.02, 3.03)	
*HLA-DRB1*1502*			1.98 (1.13, 3.40)	
*HLA-DRB1*1501*				
*HLA-DRB1*0802*				

*Odds ratio was calculated using an unconditional step-wise logistic regression analysis.

examined (i.e. *HLA-DRB1* *0405, *0803, *0901, *1502, *1501, *0802) (Table 4). Age and ANA status at the end of observation were used for the analysis. An unconditional step-wise logistic regression analysis revealed that the *HLA-DRB1* *0803 allele predisposed patients to anti-centromere antibody production (OR, 2.30, 95% CI, 1.41–3.73), while the *HLA-DRB1*0405* allele predisposed patients to anti-gp210 antibody production (OR, 1.61, 95% CI, 1.08, 2.39). Age was also a risk factor for the production of anti-centromere antibodies (OR, 1.05, 95% CI, 1.03–1.07). The *HLA-DRB1*1502* and *0901 alleles were associated with progression to clinical stage II (nonjaundice-type progression) (OR, 1.98, 95% CI, 1.13–3.40 and OR, 1.78, 95% CI, 1.02–3.03, respectively), whereas no *HLA-DRB1* allele predisposed patients to progression to clinical stage III (jaundice-type progression).

The association of *HLA-DRB1* alleles with the relative risk of ANAs for disease progression

To study the influence of *HLA-DRB1* polymorphisms on the predictive value of ANAs for disease progression, the relative risk of anti-gp210 or anti-centromere antibodies for disease progression was analyzed by a step-wise logistic regression after stratifying patients by the *HLA-DRB1* alleles that had an allelic frequency greater than 5% (i.e. *HLA- DRB1*0405, *0803, *0901, *1502, *1501, *0802*). Regardless of the *HLA-DRB1* alleles, anti-gp210 antibody seropositivity was a very strong risk factor for jaundice-type progression (OR, 48.62, 95% CI, 14.30–304.82) (Table 5). As for nonjaundice-type progression, the presence of anti-gp210 and anti-centromere antibodies showed the highest relative risk (OR, 11.50, 95% CI, 3.81–43.79 and OR, 6.89, 95% CI, 2.18–26.56, respectively) with the *HLA-DRB1*0405* allele (Table 5). Anti-centromere antibody seropositivity was also a strong risk factor for nonjaundice-type progression in patients with the *HLA-DRB1*0803* allele (OR, 5.42, 95% CI, 1.47–24.62) but not other *HLA-DRB1* alleles, including *HLA-DRB1*0901, *1502, *1501, *0802* (Table 5). In contrast, the presence of anti-gp210 antibodies was a significant risk factor for nonjaundice-type progression in patients with *HLA-DRB1*0901* (OR, 7.27, 95% CI, 1.64–40.34) and *1502* (OR, 4.35, 95% CI, 1.22–16.94) but not other *HLA-DRB1* alleles including *HLA-DRB1*0803*. Thus, the *HLA-DRB1* polymorphism determines the relative risk of ANAs for disease progression in Japanese patients with PBC.

DISCUSSION

IN THE PRESENT study, the *HLA-DRB1*0803* allele was associated with an increased susceptibility to PBC development (OR, 2.24, 95% CI, 1.48–3.41; $P = 0.0001$), which is consistent with previous reports that found a positive association between PBC susceptibility and *HLA-DRB1 *0803, *0801* and *08* in Japanese, European, and Italian populations, respectively.[17–21,24,25,28] In addition, we found for the first time that the *HLA-DRB1*0405* allele conferred susceptibility to PBC development (OR, 1.53, 95% CI, 1.11–2.11, $P = 0.005$). There have been one report which indicated the positive association between *HLA-DPB1*0501* and disease-susceptibility in Japanese patients with PBC.[23] Since *HLA-DPB1*0501* is in linkage disequilibrium with *HLA-DRB1*0405* in Japanese general population, the above association can be explained by the positive association between *HLA-DRB1*0405* and disease-susceptibility in Japanese patients with PBC.

*HLA-DRB1*0405* is known to be associated with disease susceptibility in Japanese patients with AIH.[34] Accordingly, the frequency of the *HLA-DRB1*0405* allele was significantly increased in AIH-PBC overlap patients (17/50 = 34.0%) compared to PBC patients without AIH (109/618 = 17.6%) (OR, 2.45, 95% CI, 1.29–4.47; $P = 0.007$) in the present study. However, the frequency of the *HLA-DRB1*0405* allele was also significantly increased in PBC patients without AIH compared to the general Japanese population (17.6% vs. 13.2%, OR, 1.41, 95% CI, 1.01–1.95; $P = 0.040$), indicating the significant role of *HLA-DRB1*0405* allele in the development of PBC. The accompanied autoimmune diseases such as Sjogren's syndrome, Hshimoto's thyroiditis and RA did not affect the results of positive association between *HLA-DRB1*0405* and PBC development (data not shown). This novel association between *HLA-DRB1*0405* and PBC development needs to be confirmed with a larger number of Japanese PBC patients as well as other ethnic groups.

In addition, we found for the first time that the *HLA-DRB1*1101, *1302, *1501* alleles protected against disease development in Japanese patients with PBC. Our results are compatible with studies performed in Italian and UK patients, in which *HLA-DRB1*11* and *DRB1*13* were associated with protection against disease development.[18,21,28] Interestingly, bile duct damage and portal lymphocyte infiltration are less severe in Japanese patients with chronic hepatitis C who have the *HLA-DRB1* 1302* allele.[35] Piecemeal necrosis is also less severe in patients who have the *HLA-*

Table 5 Antinuclear antibodies as a risk factor for disease progression in primary biliary cirrhosis after stratification by the *HLA-DRB1* allele

HLA-DRB1 Allele	Variables	*Odds ratio (95% confidence interval [CI]) for progression to Clinical stage II OR (95% CI)	Clinical stage III OR (95% CI)
Total	Sex	1.82 (1.04, 3.16)	
$n = 668$	Age (years)	1.04 (1.02, 1.07)	0.94 (0.91, 0.97)
	Anti-gp210-positive	3.67 (2.42, 5.58)	48.62 (14.30, 304.82)
	Anti-centromer-positive	2.41 (1.56, 3.73)	
**0405*	Sex		
$n = 126$	Age		0.86 (0.75, 0.95)
	Anti – gp210 – positive	11.50 (3.81, 43.79)	6.03×10^6 (8.85,∞)
	Anti-centromere-positive	6.89 (2.18, 26.56)	
**0803*	Sex		
$n = 89$	Age	1.06 (1.00, 1.15)	0.87 (0.74, 0.98)
	Anti-gp210-positive		1.56×10^7 (11.24,∞)
	Anti-centromere-positive	5.42 (1.47, 24.62)	
**0901*	Sex	36.38 (5.91, 383.94)	
$n = 89$	Age	1.13 (1.06, 1.24)	
	Anti-gp210-positive	7.27 (1.64, 40.34)	6.84×10^6 (5.03,∞)
	Anti-centromere-positive		
**1502*	Sex		
$n = 81$	Age	1.05 (1.01, 1.11)	
	Anti-gp210-positive	4.35 (1.22, 16.94)	14.38 (1.80, 303.94)
	Anti-centromere-positive		
**1501*	Sex		
$n = 46$	Age		
	Anti-gp210-positive		8.82×10^{12} (20.03,∞)
	Anti-centromere-positive		
**0802*	Sex		
$n = 37$	Age		
	Anti-gp210-positive		7.67×10^6 (3.97, ∞)
	Anti-centromere-positive		
Others	Sex		
$n = 200$	Age	1.04 (1.00, 1.08)	
	Anti-gp210-positive	4.73 (2.21, 10.43)	19.31 (3.07, 388.96)
	Anti-centromere-positive		

*Odds ratio was calculated using an unconditional step – wise logistic regression analysis.

*DRB1*1101* allele.[35] These results may possibly indicate that weaker bile duct damage, portal lymphocyte infiltration and piecemeal necrosis might be associated with a protective role for the *HLA-DRB1 *1101* and **1302* alleles in the development of PBC. We also found for the first time that *HLA-DRB1 *1502* and **0901* are risk factors for nonjaundice-type progression. Interestingly, the degree of piecemeal necrosis and portal lymphocyte infiltration is more severe in chronic hepatitis C patients with the *HLA-DRB1*1502* allele.[35] It might be possible that stronger piecemeal necrosis and portal lymphocyte infiltration may also play a significant role in PBC progression in patients with the *HLA-DRB1 *1502* allele.

As for the influence of *HLA-DRB1* polymorphisms on ANA production, we found for the first time that *HLA-DRB1*0803* and *HLA-DRB1*0405* are significant risk factors for the production of anti-centromere and anti-gp210 antibodies, respectively, in Japanese patients with PBC. It is well known that autoantibody production is influenced by specific HLA class II genes; for example, *HLA-DRB1*0803* is associated with anti-Ro/La and anti-topoisomerase I antibody production in Japanese patients with systemic lupus erythematosus (SLE) and systemic sclerosis (SSc), respectively, while *HLA-DRB1*0405* is associated with the production of anti-RNA polymerase I/III and anti-citrullinated peptides

(CCP) antibodies in Japanese patients with SSc and rheumatoid arthritis (RA), respectively.[36–42] Further, the HLA polymorphism is associated with higher titer of autoantibodies and disease-progression in RA and SSc.[37–40] However, the mechanisms underlying these preferential production of autoantibodies and disease-progression in patients with a certain *HLA-DRB1* allele remain to be elucidated.

In addition, we presented for the first time the association of *HLA-DRB1* polymorphism with the relative risk of ANAs for disease progression in PBC. *HLA-DRB1*0405*, *0803, *0901, *1502, *1501, and *0802 alleles constitute 18.9%, 13.3%, 13.3%, 12.1%, 6.9% and 5.5%, respectively, of the total alleles in Japanese patients with PBC. Stratification of PBC patients by these *HLA-DRB1* alleles revealed that the relative risk of ANAs for disease progression to the nonjaundice stage was significantly influenced by different *HLA-DRB1* alleles, whereas the relative risk of anti-gp210 antibody seropositivity for jaundice-type progression was very strong regardless of differences in the *HLA-DRB1* alleles. The presence of anti-gp210 and/or anti-centromere antibodies in late stage PBC with nonjaundice and jaundice-type progression was more than 95% in patients with the *HLA-DRB1*0803* allele (20/21 = 95.2%) and **0405* allele (36/37 = 97.3%), indicating that PBC patients who have the *HLA-DRB1 *0803* or **0405* alleles and are seronegative for both anti-centromere and anti-gp210 antibodies are in the lowest risk group for PBC disease progression. For these patients, the risk of progression is less than 5%. For other *HLA-DRB1* alleles including *HLA-DRB1*0901*, **1502* and **1501*, anti-gp210 and/or anti-centromere antibody seropositivity was less than 70% during late stage PBC (17/29 = 65.5%, 6/11 = 68.8%, and 22/32 = 54.5%, respectively), indicating that PBC patients with the *HLA-DRB1*0901*, **1502* or **1501* alleles who are negative for both anti-centromere and anti-gp210 antibodies are still at risk (more than 30%) to progress to late stage disease.

In conclusion, our results indicate that *HLA-DRB1* polymorphisms are significantly associated with multiple steps in disease development, progression, and ANA production, and in determining the relative risk of ANAs for PBC disease progression. Since the predictive value of anti-gp210 and anti-centromere antibodies for disease progression was the highest in patients with the *HLA-DRB1*0405* and **0803* alleles, which are the most prevalent (one-third) among Japanese patients with PBC, identifying the *HLA-DRB1* allele in PBC patients is useful to predict the long-term outcome of PBC, which includes at least three different types of progression: no progression, nonjaundice-type progression and jaundice-type progression. Further, stratifying patients by *HLA-DRB1* polymorphisms would be useful to identify unknown genetic and serological biomarkers that could contribute to disease development and progression in PBC. Identifying these factors is the focus of future investigations.

ACKNOWLEDGEMENTS

THIS WORK WAS supported by Grants-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare of Japan, Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science, and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and the Program of Founding Research Centers for Emerging and Reemerging Infectious Diseases, MEXT Japan.

REFERENCES

1 Scheuer PJ. Primary biliary cirrhosis. *Proc R Soc Med* 1967; 60: 1257–60.
2 Rubin E, Schaffner F, Popper H. Primary biliary cirrhosis. *Am J Pathol* 1965; 46: 387–407.
3 Leung PSC, Coppel RL, Ansari A, Munoz S, Gershwin ME. Antimitochondrial antibodies in primary biliary cirrhosis. *Semin Liver Dis* 1997; 17: 61–9.
4 Courvalin JC, Worman HJ. Nuclear envelope protein autoantibodies in primary biliary cirrhosis. *Semin Liver Dis* 1997; 17: 79–90.
5 Invernizzi P, Selmi C, Ranftler C, Podda M, Wesierska-Gadek J. Antinuclear antibodies in primary biliary cirrhosis. *Semin Liver Dis* 2005; 25: 298–310.
6 Makinen D, Fritzler M, Davis P, Sherlock S. Anti-centromere antibodies in primary biliary cirrhosis. *Arthritis Rheum* 1983; 26: 914–17.
7 Invernizzi P, Podda M, Battezzati PM *et al.* Autoantibodies against nuclear pore complexes are associated with more active and severe liver disease in primary biliary cirrhosis. *J Hepatol* 2001; 34: 3g66–72.
8 Muratori P, Muratori L, Ferrari R *et al.* Characterization and clinical impact of antinuclear antibodies in primary biliary cirrhosis. *Am J Gastroenterol* 2003; 98: 431–7.
9 Nakamura M, Shimizu-Yoshida Y, Takii Y *et al.* Antibody titer to gp210-C terminal peptide as a clinical parameter for monitoring primary biliary cirrhosis. *J Hepatol* 2005; 42: 386–92.
10 Wesierska-Gadek J, Penner E, Battezzati PM *et al.* Correlation of initial autoantibody profile and clinical outcome in primary biliary cirrhosis. *Hepatology* 2006; 43: 1135–44.

11 Nakamura M, Kondo H, Mori T *et al.* Anti-gp210 and anti-centromere antibodies are different risk factors for the progression of primary biliary cirrhosis. *Hepatology* 2007; **45**: 118–27.
12 Nakamura M, Komori A, Ito M *et al.* Predictive role of anti-gp210 and anti-centromere antibodies in long-term outcome of primary biliary cirrhosis. *Hepatol Res* 2007; **37**: S412–419.
13 Nishimura Y, Oiso M, Fujisao S *et al.* Peptide-based molecular analysis of HLA class II-associated susceptibility to autoimmune diseases. *Intern Rev Immunol* 1998; **17**: 229–62.
14 Shiina T, Inoko H, Kulski JK. An update of the HLA genomic region, locus information and disease associations. *Tissue Antigens* 2004; **64**: 631–49.
15 Gregersen PK, Behrens TW. Genetics of autoimmune diseases-disorder of immune homeostasis. *Nat Rev Genet* 2006; **7**: 917–28.
16 Juran BD, Lazaridis KN. Genetics and genomics of primary biliary cirrhosis. *Clin Liver Dis* 2008; **12**: 349–65.
17 Underhill J, Donaldson P, Bray G, Doherty D, Portmann B, Williams R. Susceptibility to primary biliary cirrhosis is associated with the HLA-DR8-DQB1*0402 haplotype. *Hepatology* 1992; **16**: 1404–8.
18 Begovich AB, Klitz W, Moonsamy PV *et al.* Genes within the HLA class II region confer both predisposition and resistance to primary biliary cirrhosis. *Tissue Antigens* 1994; **43**: 71–7.
19 Wassmuth R, Depner F, Danielsson A *et al.* HLA class II markers and clinical heterogeneity in Swedish patients with primary biliary cirrhosis. *Tissue Antigens* 2002; **59**: 381–7.
20 Donaldson P, Agarwal K, Craggs A, *et al.* HLA and interleukin 1 gene polymorphisms in primary biliary cirrhosis: associations with disease progression and disease susceptibility. *Gut* 2001; **48**: 397–402.
21 Donaldson PT, Baragiotta A, Heneghan MA *et al.* HLA class II alleles, genotypes, haplotypes and amino acids in primary biiary cirrhosis: a large-scale study. *Hepatology* 2006; **44**: 667–74.
22 Mella JG, Roschmann E, Maier K-P, Volk BA. Association of primary biliary cirrhosis with the allele HLA-DPB1*0301 in a German population. *Hepatology* 1995; **21**: 398–402.
23 Seki T, Kiyosawa K, Ota M *et al.* Association of primary biliary cirrhosis with human leukocyte antigen DPB1*0501 in Japanese patients. *Hepatology* 1993; **18**: 73–8.
24 Onishi S, Sakamaki T, Maeda T *et al.* DNA typing of HLA class II genes; DRB1*0803 increases the susceptibility of Japanese to primary biliary cirrhosis. *J Hepatol* 1994; **21**: 1053–60.
25 Mukai T, Kimura A, Ishibashi H *et al.* Association of *HLA-DRB1*0803* and *1602 with susceptibility to primary biliary cirrhosis. *Int Hepatol Comm* 1995; **3**: 207–12.
26 Invernizzi P, Battezzati PM, Crosignani A *et al.* Peculiar HLA polymorphisms in Italian patients with primary biliary cirrhosis. *J Hepatol* 2003; **38**: 401–6.
27 Liu HY, Deng AM, Zhou Y, Yao DK, Zhong RQ. Analysis of HLA alleles polymorphism in Chinese patients with primary biliary cirrhosis. *Hepatobiliary Pancreat Dis Int* 2006; **5**: 129032.
28 Invernitz P, Selmi C, Poli F *et al.* Human leukocyte antigen polymorphisms in Italian primary biliary cirrhosis: a multicenter study of 664 patients and 1992 healthy controls. *Hepatology* 2008; **48**: 1906–12.
29 Hirschfield GM, Liu X, Xu C *et al.* Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. *N Engl J Med* 2009; **360**: 2544–55.
30 Stone J, Wade JA, Cauch-Dudek K, *et al.* Human leukocyte antigen class II associations in serum antimitochondrial antibodies (AMA)-positive and AMA-negative primary biliary cirrhosis. *J Hepatol* 2002; **36**: 8–13.
31 Poupon R, Chazouilleres O, Corpechot C, Chretien Y. Development of autoimmune hepatitis in patients with typical primary biliary cirrhosis. *Hepatology* 2006; **44**: 85–90.
32 McGinnis MD, Conrad MP, Bouwens AG, Tilanus MG, Kronick MN. Automated, solid-phase sequencing of DRB region genes using T7 sequencing chemistry and dye-labeled primers. *Tissue Antigens* 1995; **46**: 173–9.
33 Kimura A, Sasazuki T. Eleventh international histocompatibility workshop reference protocol for the HLA DNA typing technique. In: Tsuji K, Aizawa M, Sasazuki T, eds. *HLA 1991 Proceedings of the Eleventh International Histocompatibility Workshop and Conference.* Oxford, UK: Oxford University Press, 1992; 397–418.
34 Seki T, Ota M, Furuta S *et al.* HLA class II molecules and autoimmune hepatitis susceptibility in Japanese patients. *Gastroenterology* 1992; **103**: 1041–8.
35 Haruna Y, Miyamoto T, Yasunami R, Kanda T, Fushimi H, Kotoh K. Human leukocyte antigen DRB1*1302 protects against bile duct damage and portal lymphocyte infiltration in patients with chronic hepatitis C. *J.* 2000;**32**: 837–42.
36 Reveille JD. The genetic basis of autoantibody production. *Autoimmun Rev* 2006; **5**: 389–98.
37 van Gaalen FA, van Aken J, Huizinga TWJ *et al.* Association between HLA class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis. *Arthritis Rheum* 2004; **50**: 2113–21.
38 Szodoray P, Szabo Z, Kapitany A *et al.* Anti-citrullinated protein/peptice autoantibodies in association with genetic and environmental factors as indicators of disease outcome in rheumatoid arthritis. *Autoimmun Rev* 2009.
39 van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Huizinga TW, Toes RE, de Vries RR. The *HLA-DRB1* shared epitope alleles are primary a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. *Arhritis Rheum* 2006; **54**: 1117–21.

40 Kuwana M, Inoko H, Kameda H *et al.* Association of human leukocyte antigen class II with autoantibody profiles, but not with disease susceptibility in Japanese patients with systemic sclerosis. *Intern Med* 1999; **38**: 336–44.
41 Kuwana M, Okano Y, Kaburaki J, Inoko H. HLA class II genes associated with anticentromere antibody in Japanese patients with systemic sclerosis (scleroderma). *Ann Rheum Dis* 1995; **54**: 983–7.
42 Reveille JD, Owerbach D, Goldstein R, Moreda R, Isern RA, Arnett FC. Association of polar amino acids at position 26 of the HLA-DQB1 first domain with the anticentromere autoantibody response in syustemic sclerosis (Scleroderma). *J Clin Invest* 1992; **89**: 1208–13.